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blockade of retinal waves results in retinogeniculate axons that develop eye-specific segregation on a local scale (Muir-Robinson et al., 2002, Huberman et al., 2002). Thus, spontaneous retinal activity may be critical for establishing large-scale topography, but later retinal activity drives refinement on a single neuron level.

The most surprising result of this study is that early blockade of spontaneous retinal activity dramatically increased receptive field size (by >30-fold!) for binocular neurons in primary visual cortex. This is particularly surprising since the distribution of binocular cells is not affected, nor is there any effect on monocular neurons in the same region of cortex. This lack of retinotopic refinement is not due to an expansion of the receptive field in the dLGN, as the authors previously demonstrated it to be normal in size following epibatidine application (Huberman et al., 2002). Any mechanistic explanation for this dramatic phenotype must (1) involve ocular dominance competition, since only cells that receive input from the two eyes are affected, and (2) occur very early in development, when thalamic axons first innervate the visual cortex. The authors present several possibilities, including a possible role for early cortical circuits involving subplate neurons, a transient population of neurons that are implicated in the formation of ocular dominance columns. Subplate neurons receive thalamic input early in development and may be critical for the maturation of cortical GABAergic circuits (Kanold and Shatz, 2006). One intriguing possibility is that this early activity instructs the development of cortical GABAergic circuits that restrict the receptive fields of binocular cells.

The two studies on visual circuitry in this issue strongly implicate a role for spontaneous retinal activity in driving synaptic refinement at retinogeniculate synapses and the forming of ODCs. However, they do not address whether the highly correlated patterns of retinal waves are instructive for these processes. Indeed, some disruptions of endogenous firing patterns disrupt eye-specific segregation while others do not, implying that some features of retinal waves may be instructive for driving map refinement (reviewed in Torborg and Feller, 2005). An alternate hypothesis is that activity is permissive, and that molecular cues dictate the detailed organization of visual maps. Though all parties agree that there is a role for both activity and molecular cues, we anticipate that the debate will be continued for many years to come.

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Promiscuous Interactions between AMPA-Rs and MAGUKs

What controls the number of AMPA receptors at excitatory synapses? MAGUKs are known to play a critical role in this process, but which ones are involved and when has been contentious. In this issue of *Neuron*, Elias et al. have elucidated the roles of three MAGUKs, PSD-95, PSD-93, and SAP-102, in the targeting of AMPA receptors to synapses in hippocampal neurons.

AMPA receptors (AMPA-Rs) mediate the vast majority of fast excitatory synaptic transmission in the brain, and regulation in their number and properties underlies major forms of synaptic plasticity (Bliss and Collingridge, 1993). There has therefore been considerable interest in determining the mechanisms that control AMPA-R trafficking to, and clustering at, synapses (Collingridge et al., 2004). One focus has been of a family of membrane-associated guanylate kinases (MAGUKs), of which four members are expressed at excitatory synapses: PSD-95, PSD-93, SAP-102, and SAP-97. However, conflicting data have emerged from these studies. For example, while overexpression of PSD-95 was found to increase AMPA-R-mediated synaptic transmission at hippocampal synapses (Schnell et al., 2002), targeted truncation of PSD-95 had no effect on AMPA-R-mediated synaptic transmission at these synapses (Migaud et al., 1998). Does the former result represent an overexpression artifact, or could the latter be explained by compensation in the knockout?

In the present issue of *Neuron*, Elias et al. (2006) have addressed this and related issues in a comprehensive analysis of the role of MAGUKs in AMPA-R targeting. They compare the effects of acute knockdown, using short hairpin RNAs (shRNAs) delivered by lentiviruses, with conventional knockouts of these proteins. In a particularly nice set of controls, they express the shRNAs in the corresponding knockouts to look for nonspecific effects. The conclusions they reach are clear cut and reenforce the need to interpret the results from knockout animals with great caution due to the compensation

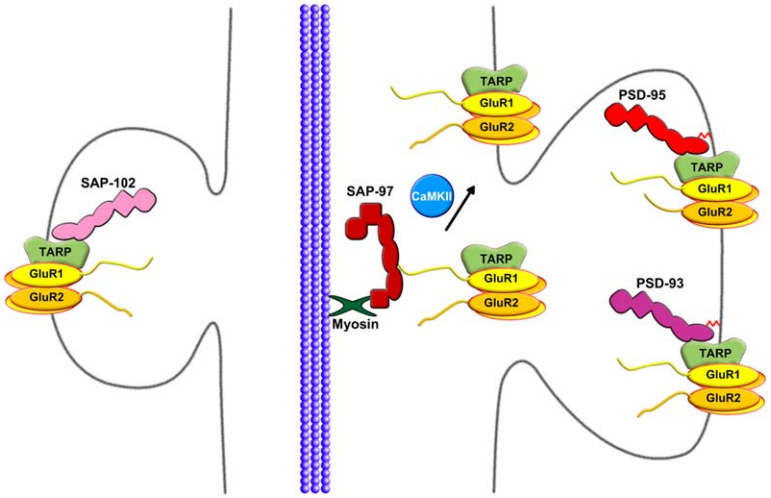

Figure 1. Regulation of Synaptic AMPA-Rs by MAGUKs

On the left-hand side is shown an immature spine where the principal MAGUK is SAP-102, which probably binds to AMPA-R subunits via TARPs (transmembrane AMPA receptor regulatory proteins). In the more mature spine (right-hand side) this role is taken over by PSD-95 and/or PSD-93, which are targeted to the membrane via their palmitoylated N termini (α isoforms). The β isoform of SAP-97 may deliver AMPA-Rs to the plasma membrane in a CaMKII-dependent manner. It may also bind to motor proteins via its L27 domain.

that can occur. They show that in immature synapses, synaptic targeting of AMPA-Rs mainly involves SAP-102. However, as animals mature, this role is taken over jointly by PSD-93 and PSD-95 (Figure 1).

Much of the interest in this study is in the detail. Acute knockdown of either PSD-95 or PSD-93 led to the removal of all AMPA-Rs, at about half of excitatory synapses in each case, probably by enabling them to diffuse to extrasynaptic loci. In contrast, in knockout animals in which ether protein had been completely eliminated, there was no effect on AMPA-R-mediated synaptic transmission. However, transmission was grossly impaired in the double knockout, suggesting that PSD-93 and PSD-95 can probably compensate for each other in knockouts. Yet the deficit in the PSD-95/PSD-93 was less than simply the additive effects of the acute knockdown of either protein alone, suggesting that there had also been compensation in this animal too. This seemed to be due to upregulation of SAP-102; acute knockdown of SAP-102 had no effect in wild-types, consistent with the major role of PSD-95 and PSD-93, but greatly reduced AMPA-R-mediated synaptic transmission in the double knockout. While acute knockdown of SAP-102 had no effect in adult wild-types, it had dramatic effects at immature synapses, at a time when the PSD-95/PSD-93 double knockout appeared normal and acute knockdown of PSD-95 was without effect. Thus, SAP-102 is the dominant MAGUK for AMPA-R trafficking early in development as well as able to partially compensate for the absence of both PSD-95 and PSD-93 in adults.

An earlier surprising observation was that manipulating expression levels of MAGUKs predominantly affected AMPA-Rs rather than NMDA-Rs, given that PSD-95 was initially identified at a binding partner of NMDA-Rs (Kornau et al., 1995). So what role do MAGUKs play in the regulation of NMDA-Rs? Although acute knockdown of either PSD-93 or PSD-95 had no effect on NMDA-R-mediated synaptic transmission, the combined knockdown of both proteins did, which is consistent with their structural role at the PSD.

So what of the role of SAP-97? Elias et al. (2006) did not address its role since their shRNA constructs, which were effective in COS-7 cells, did not work in neurons. However, SAP-97 is an interesting molecule which, unlike the other MAGUKs, can bind directly to AMPA-Rs (Leonard et al., 1998). SAP-97 binds to the PDZ domain of the GluR1 subunit and might be involved in the CaMKII-mediated targeting of AMPAs to synapses (Hayashi et al., 2000). How this occurs, however, is far from clear since deletion of the PDZ domain of GluR1 does not appear to affect AMPA-receptor-mediated synaptic transmission or plasticity (Kim et al., 2005). In another recent issue of *Neuron*, Schluter et al. (2006) have addressed the role of both SAP-97 and PSD-95 in AMPA-R targeting and synaptic plasticity. They too used viral delivery of shRNAs to acutely knock down the endogenous proteins and in an ingenious extension of this approach simultaneously expressed various isoforms of the protein of interest. They were able to show that SAP-97 can rescue AMPA-R-mediated synaptic transmission caused by knockdown of PSD-95. Therefore, yet another MAGUK has the potential to compensate for PSD-95. Interestingly, they found that although α-PSD-95 is the dominant MAGUK controlling AMPA-R number in an activity-independent manner that β-SAP-97 may regulate AMPA-R number in a CaMKII- and activity-dependent manner.

These studies raise a number of questions. For example, it was suggested that knockdown of PSD-95 and PSD-93 eliminates AMPA-Rs in largely nonoverlapping populations of synapses. So what regulates the level of expression of these MAGUKs at individual synapses? To what extent do alterations in AMPA-R number occur as a consequence of changes in synaptic NMDA-Rs that occurred under some conditions, for example the combined knockdown of PSD-95 and PSD-93? Perhaps the most interesting questions pertain to the roles that MAGUKs play in activity-dependent changes in AMPA-R number at synapses. This will no doubt form the basis of future studies, given the widely held view that these alterations may constitute a key component in the molecular basis of memory.

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Transports of Delight

Intrinsic optical signals, evoked by neural activity, provide an essentially noninvasive means of monitoring the organization of brain circuitry. A new study by Gurden et al. in this issue of *Neuron* reveals a surprising role of astrocyte glutamate transporters in generating these signals.

Neuronal activity generates small changes in the intrinsic optical properties of CNS tissue. When imaged, even through the (thinned) skull, these can be used to map which parts of the cortex, or other exposed brain area, are active (Grinvald et al., 1986). Use of this functional imaging technique has allowed the discovery of novel features of cortical organization (Bonhoeffer and Grinvald, 1991), yet the cellular basis of the signals is poorly understood, with altered light scattering, changes of hemoglobin oxygenation, and increases of blood volume all thought to contribute to the signals (Frostig et al., 1990; Aitken et al., 1999; Meister and Bonhoeffer, 2001). Despite the common assumption that it is neuronal activity that is being imaged, in this issue of *Neuron*, Gurden et al. (2006) report a surprising involvement of astrocytes in generating odor-evoked intrinsic optical signals in the olfactory bulb in vivo.

Odor stimulation leads to action potentials arriving at the olfactory receptor neuron axon terminals within the olfactory bulb (see Figure 5 of Gurden et al., 2006). Here, Ca^{2+} influx releases glutamate from the axon terminals, activating postsynaptic NMDA, AMPA, and metabotropic glutamate receptors on mitral and tufted cells (the principal output cells of the glomerulus), as well as NMDA and AMPA receptors on local interneurons. The resulting spatially localized component of the activity-evoked intrinsic optical signals was found to be inhibited, as expected, by TTX, and also inhibited when glutamate release was reduced by activating the presynaptic dopamine and $GABA_B$ receptors on the axon terminals, which are normally stimulated by local interneuron activity. Thus, action potential-evoked glutamate release initiates the intrinsic optical signals. One might imagine that activation of postsynaptic neurons, leading to downstream cell swelling, increased cell metabolism, and activation of increased blood flow would then generate the optical signals. However, Gurden et al. (2006) found that the signals were not affected by blocking AMPA and NMDA receptors, nor by blocking mGluR receptors (although, since all three receptor classes were not blocked together, this leaves open the possibility that activation of either ionotropic receptors alone or metabotropic receptors alone generates sufficient postsynaptic excitation to produce the intrinsic optical signals).

Surprisingly, applying TBOA to block glutamate transporters, which are located mainly in astrocytes, reduced the optical signals by about two-thirds. (TBOA also reduced electrically evoked field potentials by a similar factor, probably because blocking transporters leads to glutamate accumulating and desensitizing AMPA receptors). TBOA is a nonspecific blocker of all Na^+-dependent glutamate transporters. Since olfactory glomeruli express two glial glutamate transporters, GLAST and GLT1, with different spatial locations (Utsumi et al., 2001), it would be interesting to use the specific GLT1 blocker dihydrokainate to determine which transporter is the main mediator of the optical signals.

How could activation of astrocyte glutamate transporters generate intrinsic optical signals? An obvious possibility is that light scattering is altered as a result of astrocytes swelling when glutamate is taken up (Schneider et al., 1992). This swelling occurs partly because glutamate transporters take up each glutamate anion with the movement of three Na^+ and one H^+ into the cell, while one K^+ moves in the other direction on the transporter and two more K^+ will exit through ion channels to maintain charge neutrality: since H^+ is osmotically inactive, effectively, four ions move in but only three move out, and the excess ion entry will be followed by osmotic water movement. In addition, there may be water transported by the glutamate transporter itself.

Intrinsic optical signals may also be partly due to the increase of blood flow and altered hemoglobin oxygenation associated with neuronal activity and resulting metabolic activity. If this is true in the olfactory bulb, then the data of Gurden et al. (2006) will also be relevant in understanding how the functional imaging signals used in BOLD and PET experiments are generated, since these also depend on neural activity evoking an increase in blood flow. However, although astrocytes have been shown to increase blood flow in response to neural activity (Zonta et al., 2003; Takano et al., 2006), this is mediated via mGluR and AMPA receptors, which Gurden et al. (2006) show are not involved in generating the optical signals in the olfactory bulb. Furthermore, for the spatially restricted responses studied by Gurden et al. (2006), it seems that, for the 630 nm illumination wavelength used, most of the intrinsic optical signal is generated by light scattering rather than by blood-related signals (Meister and Bonhoeffer, 2001).